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Controlled-Release of Leuprolide Acetate from Polylactic Acid or Copoly(Lactic/Glycolic) Acid Microcapsules: Influence of Molecular Weight and Copolymer Ratio of Polymer

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Polylactic acid (PLA) or copoly(lactic/glycolic) acid (PLGA) microcapsules containing leuprolide acetate were prepared by an in-water drying process and the release patterns were examined *in vitro*. The release rates were extremely small from microcapsules prepared with PLA of average molecular weight 22500, and from microcapsules prepared with PLGA having average molecular weight 21200 and a copolymer ratio of 75/25 (molar ratio of lactic acid to glycolic acid). The release rate of leuprolide acetate from the microcapsules prepared with PLA of average molecular weight 6000 was relatively fast, but was still too slow to give the desired drug level over one month. Several water-soluble compounds were incorporated into microcapsules prepared with PLA of average molecular weight 22500, in an attempt to increase the release rate by the creation of aqueous channels. These compounds only induced a high initial release and failed to increase drug release. The release profile of the drug from microcapsules prepared with PLGA of average molecular weight 14000 and a copolymer ratio of 75/25 was ideal for one month's release. A small initial release was observed followed by a steady release which lasted for 35 d, and approximately followed zero-order kinetics.

Keywords——injectable controlled-release formulation; leuprolide acetate; microencapsulation; polylactic acid; copoly(lactic/glycolic) acid; in-water drying process; one-month release

Leuprolide acetate, a highly potent analogue of LH-RH (luteinizing hormone releasing hormone), when administered repeatedly to animals, leads to the suppression of gonadal steroidogenesis, and consequently, depresses the weight of the accessory sex organs.[1] Daily administration of a simple injectable solution of leuprolide acetate is now in use to treat prostate cancer, but it may be undesirable as a chronic therapy. Therefore, an alternative dosage form was sought. Injectable prolonged-release microcapsule forms composed of biodegradable polymer, polylactic acid (PLA) or copoly(lactic/glycolic) acid (PLGA), in which leuprolide acetate is entrapped were selected. These polymers have been used as carriers of controlled-release dosage forms for lipophilic drugs such as naltrexone.[2] It has also been reported that a controlled-release microcapsule system containing a hydrophilic LH-RH agonist was developed[3]; however, it was noted that some difficulty exists in the prolongation of the release of hydrophilic drugs from an injectable form.[3a] Microcapsules were prepared as described in the previous paper.[4] In that paper, it was reported that leuprolide acetate could be entrapped into PLA and PLGA microcapsules with a high entrapment ratio, but release at a constant rate for one month was not achieved.

No systematic study has appeared in the literature concerning an injectable microcapsule formulation for prolonged release of a hydrophilic drug. The purpose of this study was to create injectable microcapsules in suitable dosage form for the release of leuprolide acetate at a constant rate for one month. This was done by screening certain aqueous additives, and

polymers with different molecular weights and copolymer ratios.

Experimental

Materials——Leuprolide acetate, purified gelatin and polyvinyl alcohol were the same as described previously.[4] PLA and PLGA, which were made from D,L-forms of each monomer, were supplied by Wako Pure Chemical Ind. (Osaka). These compounds, synthesized without catalysts, were amorphous powders, and the average molecular weights were determined by gel permeation chromatography by the supplier. The average molecular weights of PLA used were 22500, 12200, and 6000, and the dispersities, which are defined the ratio of the weight-average molecular weight to the number-average molecular weight, were 1.69, 1.66, and 1.66, respectively. The average molecular weights of PLGA used were 21200 and 14000 and the dispersities were 1.66 and 1.81, respectively. The dispersity is a parameter of the distribution of molecular weight. The molecular weight distributions of PLA and PLGA would be relatively narrow, since the dispersities obtained were smaller than those reported.[5] Hereafter, specific PLA will be referred to by their average molecular weight, and abbreviated as *e.g.* PLA-22500. PLGAs will be referred to by their copolymer ratio (molar ratio of lactic acid to glycolic acid) and average molecular weight and abbreviated as *e.g.* PLGA (90/10)-21200. Glyceryl monooleate (Emulsie OL) obtained from Riken Vitamin Oil Co. (Osaka), glyceryl monocaprate from Kao Chemical Co. (Tokyo), and D-lactide from Wako Pure Chemical Ind. were used. Other chemicals were of reagent grade.

Preparation of PLA and PLGA Microcapsules——PLA and PLGA microcapsules were prepared by an in-water drying method similar to that described in the previous paper.[4] The inner water phase consisted of 495 mg of leuprolide acetate in a mixture containing 80 mg of gelatin and 0.5 ml of water maintained at approximately 60 °C. The oil phase consisted of 3970 mg of PLA or PLGA plus additives as described in Table I, in 5.5 ml of dichloromethane. The oil phase containing an additive was homogeneous and clear except in the case of 30% D-lactide. Addition of 30% D-lactide generated precipitation, but microcapsules were prepared in any event. The oil phase was gradually poured into the inner water phase under vigorous stirring with a homogenizer over a few minutes to make a w/o emulsion. The emulsion obtained was cooled to approximately 15 °C to increase the viscosities of the inner water phase and the w/o emulsion, and then poured into 400 ml of a cooled aqueous 0.1% polyvinyl alcohol solution under stirring with a turbine-shaped mixer to make a (w/o)/w dual emulsion. Increase in the viscosities of the inner water phase and the w/o emulsion generated a high entrapment ratio of leuprolide acetate into microcapsules. This is a new preparation technique to efficiently entrap a water-soluble drug, as shown in the previous paper.[4] To evaporate dichloromethane, the (w/o)/w emulsion was continuously stirred with a propeller mixer for approximately two hours. The hardened microcapsules were collected by filtration.

Determination of Leuprolide Acetate Content in Microcapsules——The leuprolide acetate in the microcapsules was determined as described previously.[4] Microcapsules (50 mg) containing leuprolide acetate were dissolved in a mixture of 10 ml of dichloromethane and 20 ml of 1/30 M phosphate buffer solution pH 6.0; leuprolide acetate extracted into the buffer solution was assayed by a high performance liquid chromatography (HPLC) procedure with an ultraviolet (UV) detector under the following conditions: column, Lichrosorb RP-18, 250 mm in length with a 4 mm i.d.; column temperature, 30 °C; mobile phase, a mixture of 100 ml of 0.25 M acetonitrile and 150 ml of methyl alcohol; flow rate, 0.7 ml/min; wavelength, 280 nm.

Leuprolide Acetate Release Studies——The leuprolide acetate released from microcapsules was determined as described in the previous paper.[4] Microcapsules (50 mg) were suspended in 10 ml of the release medium consisting of 1/30 M phosphate buffer, pH 7.0, containing 0.05% Tween-80 (Kao-Atlas, Tokyo) which was added to cause microcapsules to adapt in the medium. Since leuprolide acetate was poorly recovered from the release medium because of its instability in the buffer solution, the residual leuprolide acetate in the microcapsules was periodically determined after filtering the microcapsules through a 0.8 μm Millipore filter by the analytical method mentioned above.

Observation of Microcapsules——The shape and surface characteristics of the dried microcapsules were examined by the use of a scanning electron microscope (model JSM T-300, JEOL-Technics Co., Ltd., Japan).

Results and Discussion

Characteristics of Microcapsules

Figure 1 shows a scanning electron micrograph of the microcapsules prepared by using PLA-22500. The microcapsules were fairly spherical and the surface was smooth. The large number of micropores observed on the surface were probably generated by evaporation of dichloromethane or water. This observation is different from that of Wakiyama *et al.*,[6] who reported that micropores are generated after drug release. The appearance of microcapsules

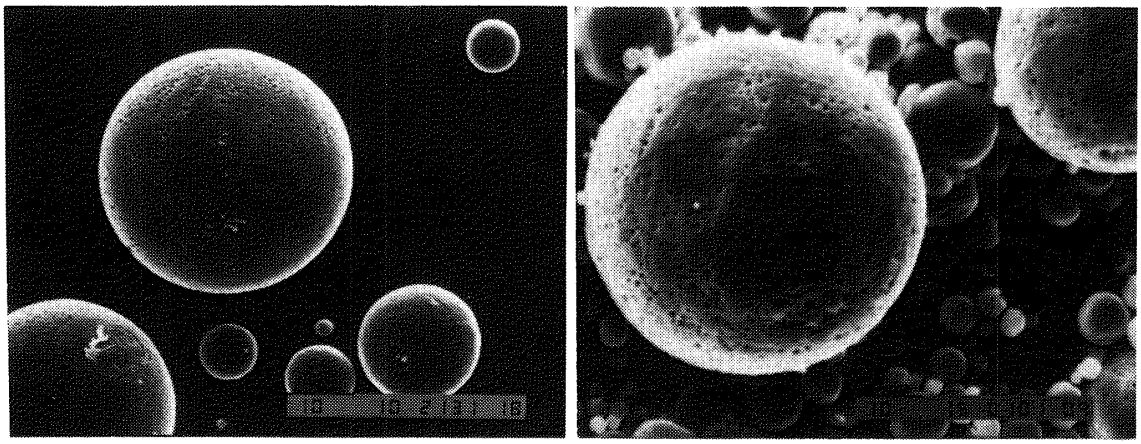

Fig. 1. Scanning Electron Photomicrographs of Microcapsules Containing 10% Leuprolide Acetate

Left-hand side: PLA-22500 microcapsules. Right-hand side: PLGA(75/25)-14000.

TABLE I. Materials Used in the Preparation of Microcapsules and the Entrapment Ratio of Leuprolide Acetate

Formulation			Entrapment ratio (%)
Polymer	Additive	Conc. (%)[a]	
PLA-22500	—	0	100
PLA-22500	Emulsie OL	2.0	78
PLA-22500	Glyceryl monocaprate	9.2	73
PLA-22500	Glyceryl monocaprate	4.8	77
PLA-22500	Glyceryl monocaprate	2.0	80
PLA-22500	Methyl *p*-hydroxybenzoate	4.8	57
PLA-12200	—	0	102
PLA-6000	—	0	73
PLGA(90/10)-21200	—	0	102
PLGA(90/10)-21200	D-Lactide	30.0	56
PLGA(90/10)-21200	D-Lactide	20.0	90
PLGA(75/25)-14000	—	0	103
PLGA(90/10)-21200/PLGA(75/25)-14000		10/100[b]	104
PLGA(90/10)-21200/PLGA(75/25)-14000		20/100	101
PLGA(90/10)-21200/PLGA(75/25)-14000		30/100	103

a) Percentage of the additive to the polymer. *b*) Mixing ratio of the two polymers.

prepared with differnt formulations was almost the same as those shown in Fig. 1.

Table I shows the types and amounts of additives used, and the entrapment ratios of the drug into the microcapsules (defined as the value of the actual leuprolide acetate content in microcapsules relative to the expected content). In spite of the use of an in-water drying method using a water-soluble drug with a solubility in water of more than 900 mg/ml, relatively high entrapment ratios were obtained. Some additives decreased the entrapment ratio of leuprolide acetate, even when the new preparation technique, reported previously,[4] was used in which the drug is easily entrapped into microcapsules at a high percentage relative to the loaded drug. The entrapment ratios of leuprolide acetate in all of the formulations containing an additive were lower than that of the formulation prepared with polymer alone.

Decrease in the entrapment ratio occurs through migration of the inner water phase to the outer water phase in the process of w/o/w emulsification. It appeared that the addition of some of the additives at relatively high concentrations readily generated a local demulsification during the process of w/o/w emulsification and allowed the inner water phase to contact the outer water phase.

Effect of Additives on the Release Rate of Leuprolide Acetate from Microcapsules

The release rate of leuprolide acetate from PLA-22500 microcapsules was too slow for one month's controlled release, as shown in Fig. 2. The release profile showed a rapid initial release, presumably of the drug located near the surface and the path-way in microcapsules, and subsequently a slow release. In order to modify the release rate of the drug, compounds which have a relatively hydrophilic portion and seem to be compatible with the polymer were incorporated into microcapsules with the aim of providing more aqueous channels. The following substances were selected: glyceryl monocaprate, glyceryl monooleate, methyl *p*-hydroxybenzoate, and D-lactide. Juni *et al.*[7] increased the release rate of aclarubicin from PLA microcapsules by using isopropyl myristate as an additive, but in this experiment, as shown in Fig. 2, additives were not effective in increasing the release rate of leuprolide acetate. The profiles of the drug remaining in formulations with additives were similar to those of the control. Any additive incorporated into the microcapsules merely increased the initial release of the drug.

Aclarubicin can dissolve in both the polymer matrix and the oily additive portion because of its lipophilicity. The drug may be released from both portions, and may be released faster from the additive than from the polymer region. However, leuprolide acetate, a water-soluble drug, can not dissolve in the polymer and the additive portion. It is considered that incorporation of an additive into the polymer matrix merely reduces its rigidity, and there fore, the drug is more readily released from the matrix at the initial stage. The effect of the inclusion of D-lactide in PLGA (90/10)-21200 microcapsules on the release rate of leuprolide acetate was studied. As shown in Fig. 3, the addition of D-lactide appeared to increase the release rate of the drug slightly and also increased the initial release. The addition of 20% D-lactide caused the drug to release more rapidly than that of 30%. The reason for this result is considered to be as follows: a part of D-lactide precipitated in the oil phase as described in the

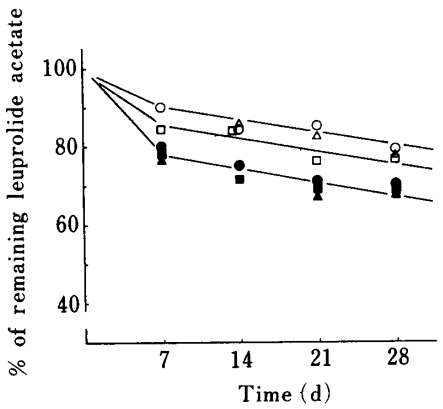

Fig. 2. Effect of Additives on the Release Profiles of Leuprolide Acetate from PLA-22500 Microcapsules

○, PLA-22500 alone (control); PLA-22500 plus ● methyl *p*-hydroxybenzoate (4.8%); ■, Emulsie OL (2.0%); □, glyceryl monocaprate (2.0%); △, glyceryl monocaprate (4.8%); ▲, glycerylmonocaprate (9.2%).

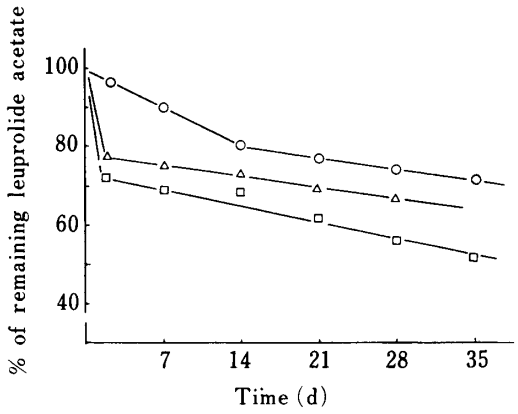

Fig. 3. Release Profiles of Leuprolide Acetate from PLGA(90/10)-21200 Microcapsules Containing D-Lactide

○, PLGA(90/10)-21200 without additive (control); △, 30% D-lactide; □, 20% D-lactide.

experimental section, and therefore, the content of D-lactide incorporated into microcapsules was similar to that in the case of 20%. However, the profile was still unsuitable for one month's controlled release. That leuprolide acetate was scarcely released from the PLGA(90/10)-21000 microcapsules shows the drug could hardly diffuse in the polymer matrix regions.

Effect of the Average Molecular Weight of Polymers and the Molar Ratio of Lactic Acid to Glycolic Acid of PLGA on the Release Rate

Since leuprolide acetate scarcely diffuses through the polymer matrix, an attempt was made to effect drug release by erosion of the polymer. The dissolution rate of the polymer after degradation by hydrolysis in the medium is faster when the molecular weight is smaller. Thus, we used polymers with various molecular weights and/or PLGA with various molar ratios of lactic acid to glycolic acid. Figure 4 shows the drug release profiles from microcapsules prepared with PLA-22500, PLA-12200, and PLA-6000. The release rate increased with decrease in the molecular weight. However, the release rate of leuprolide acetate from PLA-6000 microcapsules was still too slow for one month's controlled release. Even PLA-6000 may not be completely degraded within one month.

Subsequently, the release rate of the drug from the microcapsules prepared with PLGA(75/25)-14000 was investigated. As shown in Fig. 5, leuprolide acetate was released with an appropriate profile for over one month. A small initial release was observed followed by an approximately zero-order release which lasted for 35 d. Miller *et al.*[8] reported that the degradation of copolymers implanted in rat tissues was faster than that of the homopolymers. In this *in vitro* experiment, almost no microcapsules of PLGA(75/25)-14000 remained visible at 35 d but those of PLA-22500 were still observed in the dissolution medium at 35 d. These results indicate that leuprolide acetate was probably released from the microcapsules with monomer or oligomer by hydrolysis-erosion of the polymer wall. In order to confirm this release mechanism, the nuclear magnetic resonance (NMR) spectra of a w/o emulsion prepared with leuprolide acetate, heavy water, PLGA(75/25)-14000, and heavy chloroform were measured. A comparison of chemical shifts between an emulsion and a solution of leuprolide acetate in heavy water, showed that the δ value for the proton-singlet of histidine in the emulsion was increased. It is concluded that the proton of histidine in leuprolide acetate strongly interacts with the carbonyl group in PLGA.

Figure 1 shows an electron microphotograph of microcapsules produced with PLGA(75/25)-14000. Their appearance was similar to that of microcapsules produced with PLA-22500.

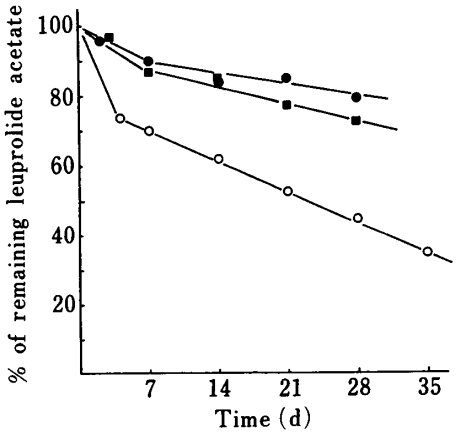

Fig. 4. Effect of Molecular Weight of PLA on Release Profiles of Leuprolide Acetate from the Microcapsules

●, average molecular weight = 22500; ■, average molecular weight = 12200; ○, average molecular weight = 6000.

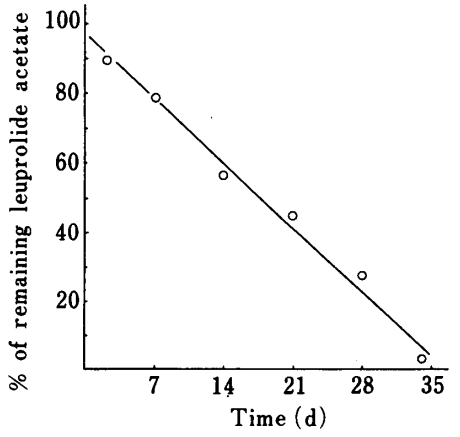

Fig. 5. The Release Profile of Leuprolide Acetate from PLGA(75/25)-14000 Microcapsules

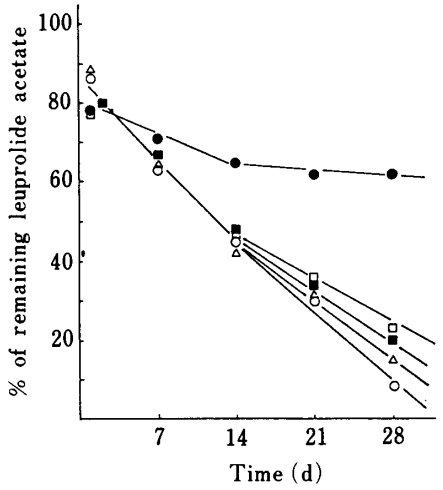

Fig. 6. Release Profiles of Leuprolide Acetate from Microcapsules Consisting of a Mixture of PLGA(90/10)-21200 and PLGA(75/25)-14000

The ratio between PLGA(90/10)-21200 and PLGA-(75/25)-14000: ●, 100/0; □, 10/100; ■, 20/100; △, 30/100; ○, 0/100.

The Release Profiles of Leuprolide Acetate from Microcapsules Prepared with a Mixture of PLGA(75/25)-14000 and PLGA(90/10)-21200

Microcapsules containing 10% leuprolide acetate were prepared with a mixture of PLGA(75/25)-14000 and 10, 20, or 30 percent PLGA(90/10)-21200. The entrapment ratios of the drug did not exhibit any significant change when the two types of polymer were combined, as shown in Table I. Figure 6 indicates that the release rates by day 14 from the microcapsules containing PLGA(90/10)-21200 at 10, 20, or 30% were substantially the same as that of the drug from the microcapsules with PLGA(75/25)-14000; the release rate after day 14 was slower from microcapsules with a higher proportion of PLGA(90/10)-21200, which would be hydrolyzed more slowly.[8] The release of the drug from PLGA(75/25)-14000 microcapsules was a little faster than that shown in Fig. 5. The reason for this is not known but the discrepancy will not influence the following discussion. It was considered that the release rate of leuprolide acetate was effected by different mechanisms up to and after day 14. Hutchinson showed that the release of peptide from a flake composition comprising PLGA proceeded by two distinct and independent mechanisms, namely first a diffusion-dependent release of the peptide from the PLGA matrix and subsequently, PLGA-degradation-dependent release.[9] It appeared that the release rate of the drug after day 14 depended upon the hydrolysis rate of the PLGA.

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